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TI Hif2 alpha, Ap-2 beta, and Et-1 cooperatively regulate  
development of the  
ductus arteriosus.  
AU Ivey, Kathryn N. [Reprint Author]; Garg, Vidu; Garcia, Joseph;  
Zhao, Feng;  
Gelb, Bruce D.; Srivastava, Deepak  
CS Univ Texas, SW Med Ctr, Dallas, TX 75230 USA  
SO Circulation, (OCT 26 2004) Vol. 110, No. 17, Suppl. S, pp. 59.  
Meeting Info.: 77th Scientific Meeting of the  
American-Heart-Association.  
New Orleans, LA, USA. November 07 -10, 2004. Amer Heart Assoc.  
CODEN: CIRCAZ. ISSN: 0009-7322.  
DT Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LA English  
ED Entered STN: 1 Dec 2005  
Last Updated on STN: 1 Dec 2005  
AB Development of the ductus arteriosus, a fetal vessel bridging the  
pulmonary and systemic vasculature, involves specification of  
highly  
contractile, oxygen-responsive vascular smooth muscle. Failure  
of this  
developmental process results in patent ductus arteriosus, the  
third most  
common congenital heart defect. We identified an individual  
with patent  
ductus arteriosus carrying a heterozygous nonsense mutation in  
the gene

encoding the endothelin-A receptor (ETA). Analysis in mouse embryos revealed that although Et-A was expressed uniformly throughout the vascular smooth muscle of the great vessels and ductus arteriosus, the gene encoding the Et-A ligand, endothelin-1 (Et-1), was uniquely confined to the smooth muscle cells of the ductus arteriosus at embryonic day (E) 13.5. A hypoxic response element upstream of Et-1 is required for hypoxic induction of Et-1 expression. We found that **Hif2** alpha, encoding a bHLH/**PAS** domain-containing hypoxia-inducible transcription factor was specifically expressed in the smooth muscle of the ductus arteriosus at E13.5 with sharp borders at the aortic and pulmonary artery junctions. Although embryonic lethality of **Hif2** alpha(-/-) embryos precluded analysis of its role in ductal development, we examined mice lacking **Tfap2** beta, a transcription factor associated with patent ductus arteriosus in humans with Char syndrome. We found that **Tfap2** beta was required for expression of both Et-1 and **Hif2** alpha in smooth muscle, but not endothelial cells of the arterial duct. Histological analysis of **Tfap2** beta(-/-) mouse embryos showed that although smooth muscle cells were present in the ductus arteriosus, they failed to maintain their highly differentiated state. Finally, **Hif2** alpha functioned as a negative regulator of **Ap-2** beta-induced transcription, suggesting a negative feedback loop that may refine the **Ap-2** beta signal during ductal development. The mechanism of negative regulation involved **Hif2** alpha disruption of sequence-specific DNA binding by **Ap-2** beta and was P300-independent. Our data, along with the requirement of **AP-2** beta for closure of the ductus arteriosus in humans, suggest that **Hif2** alpha, **Ap-2** beta, and Et-1 cooperatively regulate development of the ductus arteriosus.

DN PubMed ID: 10559262  
 TI EPAS1 trans-activation during hypoxia requires p42/p44 MAPK.  
 AU Conrad P W; Freeman T L; Beitner-Johnson D; Millhorn D E  
 CS University of Cincinnati, College of Medicine, Department of  
 Molecular and Cellular Physiology, Cincinnati, Ohio 45267-0576, USA.  
 NC HL07571 (NHLBI)  
 R37HL33831 (NHLBI)  
 R01HL59945 (NHLBI)  
 SO The Journal of biological chemistry, (1999 Nov 19) Vol. 274, No.  
 47, pp.  
 33709-13.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199912  
 ED Entered STN: 20000113  
 Last Updated on STN: 20000113  
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 AB Hypoxia is a common environmental stress that regulates gene  
 expression  
 and cell function. A number of hypoxia-regulated transcription  
 factors  
 have been identified and have been shown to play critical roles  
 in  
 mediating cellular responses to hypoxia. One of these is the  
 endothelial  
 PAS-domain protein 1 (EPAS1/HIF2-alpha/HLF/HRF). This  
 protein is 48% homologous to hypoxia-inducible factor 1-alpha  
 (HIF1-alpha). To date, virtually nothing is known about the  
 signaling  
 pathways that lead to either EPAS1 or HIF1-alpha activation.  
 Here we show  
 that EPAS1 is phosphorylated when PC12 cells are exposed to  
 hypoxia and  
 that p42/p44 MAPK is a critical mediator of EPAS1 activation.  
 Pretreatment of PC12 cells with the MEK inhibitor, PD98059,  
 completely  
 blocked hypoxia-induced trans-activation of a hypoxia response  
 element  
 (HRE) reporter gene by transfected EPAS1. Likewise, expression  
 of a  
 constitutively active MEK1 mimicked the effects of hypoxia on  
 HRE reporter  
 gene expression. However, pretreatment with PD98059 had no  
 effect on  
 EPAS1 phosphorylation during hypoxia, suggesting that MAPK  
 targets other  
 proteins that are critical for the trans-activation of EPAS1.  
 We further

show that hypoxia-induced trans-activation of EPAS1 is independent of Ras.

Finally, pretreatment with calmodulin antagonists nearly completely

blocked both the hypoxia-induced phosphorylation of MAPK and the EPAS1

trans-activation of HRE-Luc. These results demonstrate that the MAPK

pathway is a critical mediator of EPAS1 activation and that activation of

MAPK and EPAS1 occurs through a calmodulin-sensitive pathway and not

through the GTPase, Ras. These results are the first to identify a

specific signaling pathway involved in EPAS1 activation.